Population Structure and Fecundity in the Putative Sterile Shrub, *Grevillea rhizomatosa* Olde & Marriott (Proteaceae)

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Grevillea rhizomatosa Olde & Marriott (Proteaceae) is a threatened species of shrub known only from 12 populations within a 7 x 8 km area within Gibraltar Range and Washpool National Parks, northern New South Wales, Australia. Prior to this study it was believed that the species only reproduced from rhizomatous suckers as seed and fruit were never detected in the wild. A concern for the reproductive and evolutionary potential of the species in the event of a catastrophic disturbance was the basis for an investigation into the reproductive ecology of *G. rhizomatosa*. Such an event occurred in October 2002 with an intense wildfire affecting most of the populations. Five populations were studied in detail for demography and fecundity prior to this fire and two populations were resurveyed in August 2005. In 2000, 916 individual stems were recorded across these populations and only small to large shrubs were found; no seedlings were recorded. Post-fire response was documented in two populations where plants were found to be resprouting and suckering from underground stems. In the pre-fire surveys of 2000 and 2001 flowering occurred in all populations, but since the fire of October 2002 flowering has only occurred in unburnt habitats. Flowers on shrubs in two of the five populations failed to produce fruit, but low fruit-set (7-13% of flowers) occurred in three populations. Seeds collected from two populations (n = 14) were tested for viability using tetrazolium chloride and were 100% viable. Ramets were detected in all populations and resprouting from underground stems was observed after wildfire. This is the first record of viable seed in this species and fertile populations require specific management to prevent loss of fertile plants. Loss of fertile plants could occur if repeated burning selects for vegetative reproduction and sterile plants.

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INTRODUCTION

Grevillea is one of the most successfully dispersed groups within the Proteaceae. An estimated 357 species occur variously in temperate, arid and tropical ecosystems throughout Australia with species also found in New Caledonia (3 endemic species), New Guinea (3 species, 1 endemic) and Sulawesi (1 endemic species) (Makinson 2000). About 14% of species have the capacity to reproduce asexually through vegetative reproduction, although the majority of these species combine both asexual and sexual reproduction (Makinson 2000; Makinson, unpub. data). An exception to this may be *Grevillea rhizomatosa*, which is described, by Olde and Marriot (1994) as sterile and an obligate clonal species with ramets produced from stem suckering. *Grevillea rhizomatosa* is restricted to Washpool and Gibraltar Range National Parks and is listed as a vulnerable species at both a State and Federal level (*Threatened Species Conservation Act 1995* (NSW), *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth)).

The occurrence of clonality in rare and threatened plants can complicate the conservation of such species (e.g. Sydes and Peakall 1998) because ramet reproduction may cause population sizes to be overestimated (Ellstrand and Roose 1987). Moreover much of the genetic variation may exist among populations rather than within, thereby requiring all populations to be actively conserved. The clonal syndrome may be disadvantageous to species if low to nil genetic diversity is combined with sterility (e.g. *Lomatia tasmanica*, Lynch et al. 1998). This can make such species highly susceptible to extirpation, as all individuals in the population are likely to respond in a uniform fashion to a stochastic event (e.g. disease, fire). In addition, the chances of extirpation are exacerbated if populations are small and fragmented but simultaneously impacted upon by major disturbance events such as wildfire.

The absence or poor seed production in populations can have many causes that may include ecological deficiencies (e.g. pollinator and/or pollen limitation; fruit predation, e.g. Hampe 2005; Vesprini and Galetto 2000) nutrient shortages (e.g. Drenovsky and Richards 2005) and innate sterility mechanisms (Pandit and Babu 2003). As a first stage to understanding fecundity in *Grevillea rhizomatosa*, we investigated the distribution of populations with special reference to their fertility and post-fire response.

METHODS

Species distribution

Specimen data for *Grevillea rhizomatosa* from the New England Herbarium and flora survey data from the NSW-NPWS were collated and mapped resulting in 22 potential populations. Seventeen of these locations were relocated in the field and visited during August and November 2000. This preliminary work showed that the species is found in at least 12 populations in an area 8 km x 7 km in northern NSW (Fig. 1).

Population and habitat description

Five populations spanning the range of *Grevillea rhizomatosa* were chosen for further study (Fig. 1). Sites were selected based on the parameters of population size (at least 25 individuals) and range so that reproductive outputs could be compared across the extremes of the species' distribution. One study site occurred in Washpool National Park and the remainder in Gibraltar Range National Park. The selected populations were Washpool National Park (Wash), Mulligan's Hut (MHut), Dandahra Trail (Dand), M[c]Climonts Swamp (Swamp), and Murrumbooee Cascades (Cascade) (Fig. 1). Fieldwork was conducted between August and November 2000 at Cascade, MHut and Dand, with the majority of work undertaken (in all populations) between March and September 2001 with follow up work in June to August 2005.

Demography

<u>2000-2001</u>

Plants do not flower every year and ramets occur in all populations so plants were scored as seedlings (< 10 cm height, with no obvious rhizomatous connections) or as a combined class called juvenile/adult. A plant was scored as an individual if growing as a single stem or as a multi-stem plant (on the proviso that the multi-stems were grouped within a 5 cm basal diameter). Plants with more than 5 cm between them were classed as separate individuals although it is possible that they exist as ramets. In each population a maximum of 100 individuals was examined and in populations of less than 100 individuals, all were measured.

<u>2005</u>

Plant response to the October 2002 fire was scored in Wash and Dand in August 2005. In each population 25-50 individuals were measured for height and autonomy (seedling or resprouter/sucker).

Fecundity

To quantify the extent of sexual reproduction in populations, flowers on each of 10 plants were tagged in Cas, Dand, MHut and Wash over two flowering seasons (1999-2000 and 2000-2001, Dand and Cascade; and 2000-2001 MHut, Wash). Flowers open to all pollinators were tagged and then monitored for fruit-set over the 2000 and 2001 flowering season (see Table 1 for sample sizes). Bags were placed over developing fruits to reduce fruit-loss. Data were pooled across seasons. The Swamp population was not used for fecundity experiments because of time restrictions, but plants in this population were extensively searched for fruit production over the two flowering seasons.

Seed viability

Seeds were encountered only infrequently during fieldwork (see below). Seven seeds were obtained from each of three individuals at Cas and Wash. We checked the viability of seeds using a 48-hour soaking solution of a 1.0% solution of 2,3,5-triphenyl-tetrazolium chloride (Scott and Gross 2004). Seven of these seeds were killed by boiling and used as a control. Seed were dissected and scored as viable if they stained bright pink. Non-viable seeds do not stain (Lakon 1949).

RESULTS

Population and habitat description

Grevillea rhizomatosa was only found growing on low-nutrient lithosols derived from Dandahra Granite complex within Gibraltar Range and Washpool National Parks.

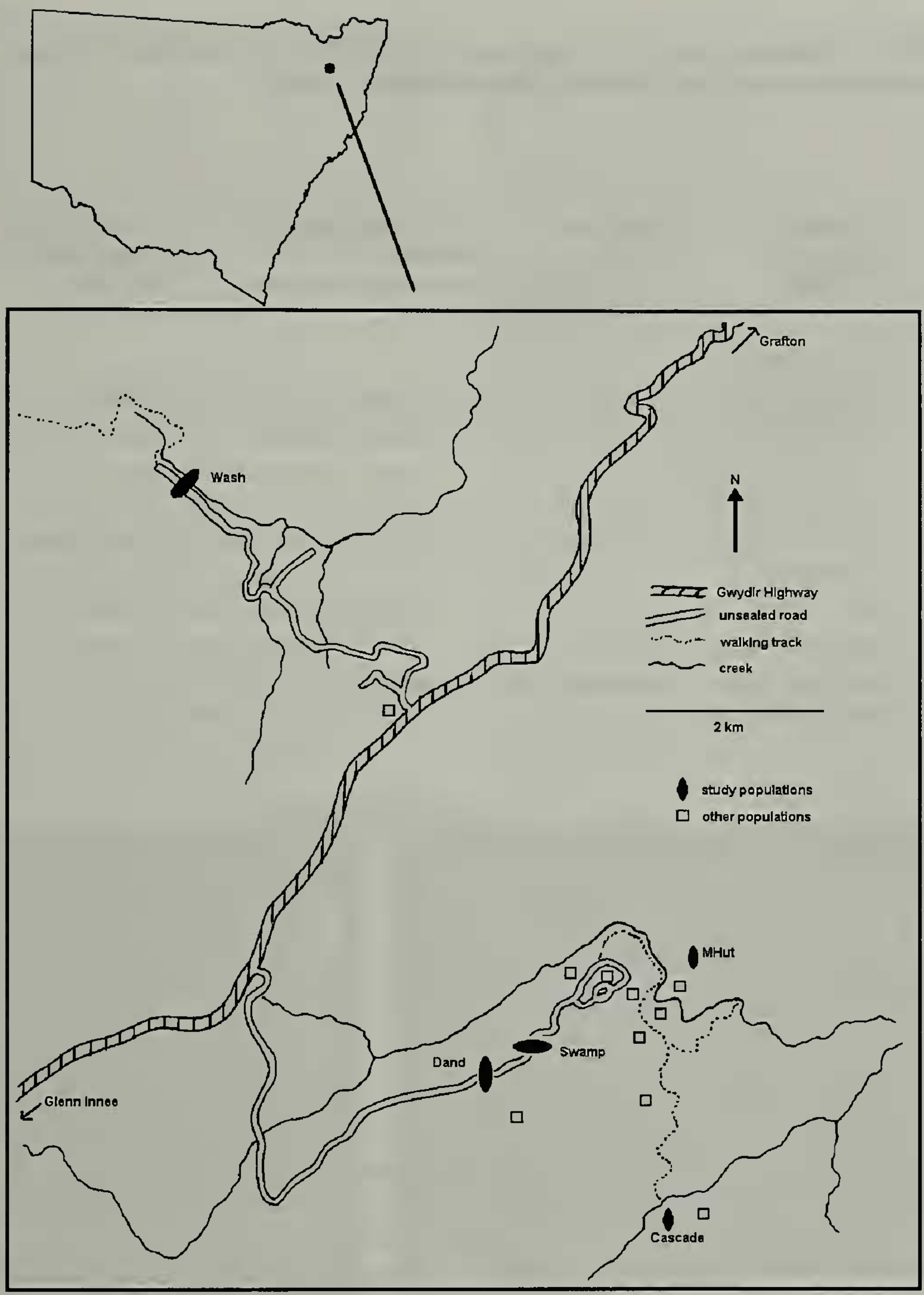

Figure 1. Distribution of *Grevillea rhizomatosa* plants in Gibraltar Range and Washpool National Parks.

<u>Washpool National Park (Wash)</u>

The northern most population of *Grevillea rhizomatosa* is located in the Washpool National Park (29° 28' 03"S, 152° 18' 18"E) at 870 m ASL. The topography is mid slope with a north-eastern aspect. The soil substrate is a deep to shallow sandy loam derived from leucogranite granite. The vegetation is tall open forest dominated by *Eucalyptus campanulata* and *E. cameronii*. Associated species include *Banksia integrifolia* subsp. *monticola*, *Pultenaea* sp. B, *Acacia nova-anglica*. *Grevillea rhizomatosa* grows in linear strips along the North and South sides of Moogem Road; at least 200 individuals grow south of the road and at least 25 scattered individuals occur to the north between the road and the Dandahra Gully. Fire records at 1 July 2002 show this area to the north of Moogem Road had not been burnt since 1968, whereas south of Moogem Road was burnt in 1988. The population on the southern side of the road was also extensively burnt in October 2002 and dense resprouting was observed in July 2005.

<u>Mulligan's Hut Camping Area (MHut)</u>

MHut is located 200 m north east of Mulligan's Hut along the world heritage

Table 1. Population locations, demography and fertility for *Grevillea rhizomatosa* from Washpool and Gibraltar Range National Parks.

Site	Latitude longitude altitude	Number of plants (% seedlings)	% fruit production (number of flowers treated over 10 plants)	% seed viability (n = number of seed treated)
Wash	29° 28' 03"S 152° 18' 18"E 870 m ASL	c. 225 (0)	10.19 (206)	100% (7)
MHut	29° 31' 00"S 152° 21' 39"E 910 m ASL	c. 250 (0)	0 (62)	-
Dand	29° 31' 42"S 152° 20' 30"E 980 m ASL	c. 250 (0)	7.08 (367)	-
Swamp	29° 31' 58"S 152° 20' 27"E 960 m ASL	165 (0)	not quantified but none observed from 2000-2005	-
Cas	29° 32' 37"S 152° 21' 29"E 830 m ASL	41 (0)	13.35 (337)	100% (7)

walking track (Table 1). Two hundred and fifty individuals of *Grevillea rhizomatosa* were found in this area. The topography is mid-slope with a south-western aspect. Shallow to skeletal sandy granitic soils occur at the site, with most plants growing between granite boulders. The vegetation is an open woodland with a dense shrub layer; the dominant tree species associated with this community include *Eucalyptus olida*, *E. pyrocarpa*, and *E. planchoniana*. Dominant understorey species include *Leptospermum trinervium*, *Pultenaea* sp. B, *Persoonia rufa*, *Banskia spinulosa*. Ground cover species include *Platysace ericoides*, *Caustis flexuosa*, *Bossiaea scortechinii*, *Xanthorrhoea johnsonii*, and *Lomandra longifolia*. The NPWS database indicates the area was burnt in 1964 and possibly in 1988. The population was not burnt in the October 2002 fires.

Dandahra Trail (Dand)

Grevillea rhizomatosa grows on both sides of the Dandahra Trail into Mulligan's Hut (Table 1). Most plants (200 stems) grow south of the Dandahra trail, with only some 50 individuals growing to the north. Shallow sandy granitic soils occur at the site. Some plants grow between granite boulders. Dominant tree species include *E. olida* and *E. cameronii*. Common shrubs are *Pultenaea* sp. B, *Persoonia rufa*, and *Acacia obtusifolia*. Groundcover species include *Platysace ericoides*, *Caustis flexuosa*, and *Bossiaea scortechinii*. NPWS fire history for the area shows fire in 1964 and 1988. The area was intensively burnt in October 2002.

McClimonts Swamp (Swamp)

The Swamp population is located approximately 500 m down-slope from Dand (Table 1). A population of 165 individuals occurs in linear strips adjacent to the road. Soil substrate, vegetation, and fire history are similar to those described for Dand.

Murrumbooee Cascades (Cascade)

Cascade is the southernmost *G. rhizomatosa* population detected in this study (Table 1). A small population of 41 individuals occurs on north and south ridges dissected by a drainage line. Soils are shallow to skeletal and of granitic derivation, as described previously. *Eucalyptus radiata* subsp. *sejuncta* is present along the creek, with *E. olida* and *E. cameronii* on the ridges. Dominant shrubs include *Leptospermum trinervium*, *Dillwynia phylicoides,* and *Hakea laevipes* subsp. *graniticola*. The area was burnt in 1964 and 1988. The October 2002 fires burnt the northern half of this population.

Demography

2000-2001

No seedlings were detected during the study. All plants were greater than 10 cm in height and most (c. 80%) appeared to be connected to nearby plants, as evidenced by plants growing in lines from larger plants and as confirmed from occasional excavations (Figure 2a-c). At MHut plants are large (0.5-1.20 m tall x c. 0.5-1.40 m wide) and many are connected underground by their stems. Large granite boulders partition this population into well-defined clumps. Flowering occurred in all populations in all years although not all plants flowered every year.

2005

No seedlings were found in the fire-recovering communities of Wash and Dand. The mean plant height of *Grevillea rhizomatosa* in the burnt habitat at Wash was 48.05± 3.81 cm (n = 43), which was considerably shorter than the few plants that escaped the fire on the northern side of the road (mean height = 108.89 ± 23.99 cm, n=7). The unburnt plants flowered in 2004 and 2005, whereas the burnt plants did not. At Dand the recovering population had a mean height of 47.64 ± 2.58 cm (n=51) in August 2005. In 2004 and 2005 flowering was only detected on unburnt individuals at Wash and in the unburnt population of MHut.

Fecundity

Fruits were only detected in Wash, Dand and Cas (Table 1, Figure 3a, 2b). Flowers have two ovules, but fruits mainly contained one seed. Fruit was recorded on each of the 10 survey plants in Wash and Cas and on eight of the 10 survey plants in

Figure 2. (a) Subterranean reprouting from a plant in Wash August 2005, (b). rhizomatous connections between small plants at Wash August 2005, (c) rhizomatous growth in *G. rhizomatosa* (scale bar = 100 mm).

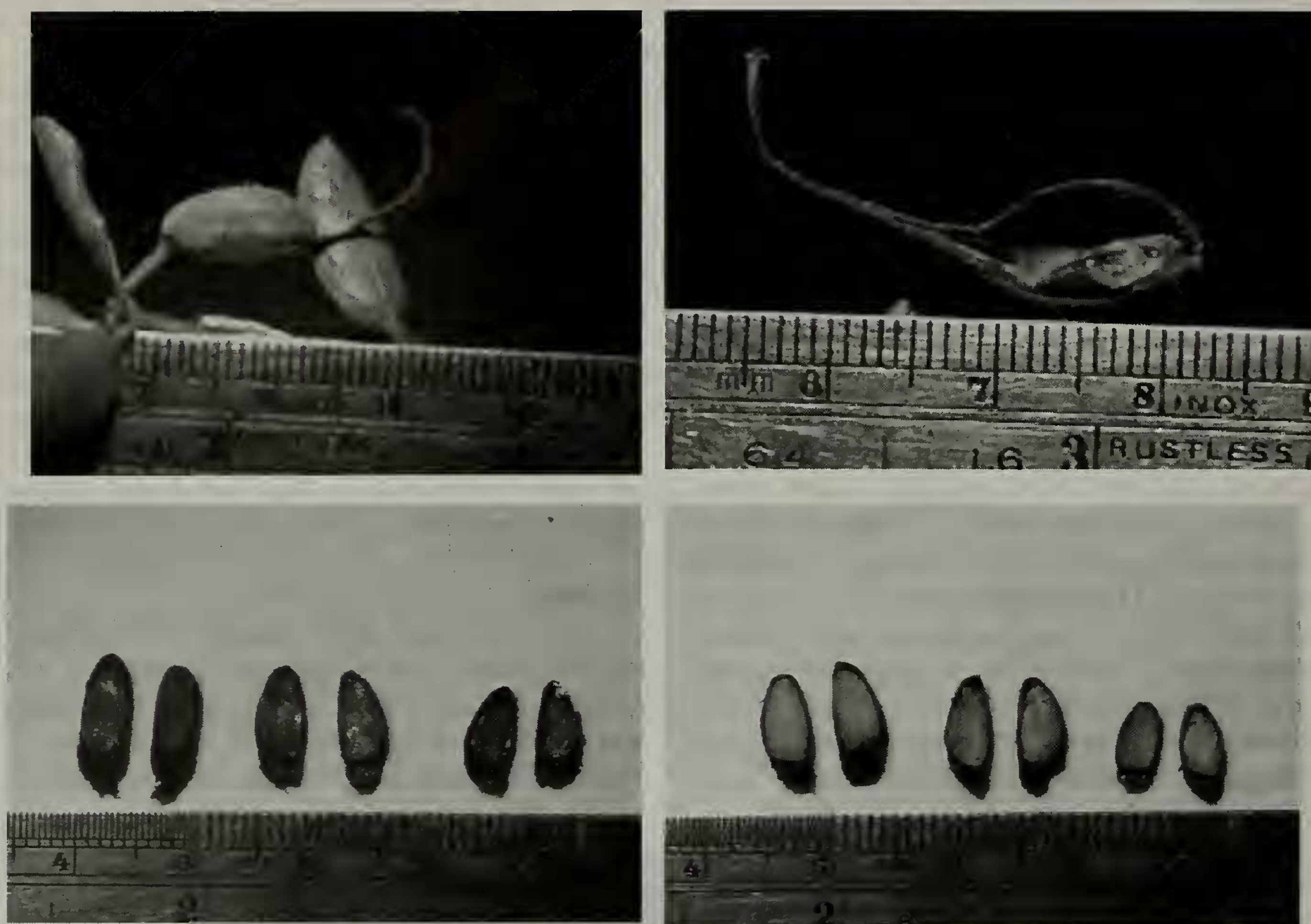

Figure 3. (a) & (b) fruits of *G. rhizomatosa* (scale bars = 10 mm), viable (c) and inviable (d) seed of *Grevillea rhizomatosa* from Wash and Cas populations. Scale bar = 10mm.

Dand. Although not quantified, there were, however, many plants that did not produce fruit in the fertile populations. No seed was produced from tagged flowers at MHut and no fruit were ever found in any season at Swamp during cursory observations.

Seed Viability

Seed collected from Wash and Cas (n = 14) and treated chemically with tetrazolium were 100% viable (Figures 3c, 3d) and controls were unviable (n = 7).

DISCUSSION

This is the first time that seed has been found on individuals of *Grevillea rhizomatosa.* Prior to our work the species was thought to be sterile and obligately clonal (Olde and Marriott 1994; Makinson 2000). Within Gibraltar Range and Washpool National Parks all five study populations of *Grevillea rhizomatosa* contained clonal individuals with all plants in two populations failing to produce fruit on any flowering plant. Seedlings were never encountered, even after fire had burnt populations containing fruit-bearing plants. These three fertile populations (Wash, Dand and Cascades) are widely separated and thus valuable for the conservation of the species. Natural fruit-set was low (< 0.14 fruit to flower ratio) but higher than that found in other species of *Grevillea* (e.g. 0.015–0.096 fruit to flower ratio at maturation, Hermanutz et al. 1998).

Not all individuals flowered every year and after the hot fires of October 2002 flowers have not been initiated on recovering individuals in Wash, Dand, Swamp or Cascade as of August 2005. Instead the species in these populations has recolonised areas by resprouting from stem bases (Fig. 2a) and from the advent of new suckers (Fig. 2b and 2c). It may be that the release from flowering allows resources to be redirected for vegetative reproductions and that clonality is selectively favoured in this irregular flower producer. This has major ramifications for the genetic stucture of populations such that near neighbours are likely to be genetically identical, which in turn may promote inbreeding when flowering occurs in populations.

Where clonality coexists with sexual forms it may provide populations with a flexible response to variable habitat or resource abundance and allow the transfer of resources among ramets. In habitats where large resource-reserves are required to initiate new growth, clonality may provide a more secure investment than seed-set alone. Clonal plants with pronounced vegetative reproduction can have lower rates of local extinction in nutrient-poor ecosystems than plants without pronounced vegetative reproduction (Fischer and Stöcklin 1997). Indeed the correlation that clonal plants are often found on nutrient-poor soils (see Fischer and van Kleunen 2002) may, in part, explain why Australia, with nutrient-poor soils, seems to have so many threatened species that are clonal (Gross, unpub. data).

The regenerative capacity of clonal growth also affords ramets increased longevity. Tyson et al. (1998) for example, found a clonal mallee eucalypt to be at least 900 years old, much older than the usual age of single stemmed eucalypts. Moreover, Smith et al. (2003) estimate from radial growth rates in *Eucalyptus curtisii* that some clones may be between 4000 and 9000 years old. The population at MHut is comprised of at least 250 large, sterile shrubs that are nestled among granite boulders. Within this population the lateral spread of plants is restricted by boulders encircling clumps, suggesting that plant clumps may not be of recent origin.

Management of *Grevillea rhizomatosa* should especially focus on the fertile populations of Wash, Dand and Cas. Of concern is the promotion of suckering in post-fire habitats, where plants can form thickets. If this is combined with sterility then seedling establishment of fertile individuals may be disadvantaged. Our work has shown that plants do not flower in the first three seasons post-fire and thus further observations are required to determine the optimal fire interval. In addition, the reasons for an absence of fruit-set in some individuals of *Grevillea rhizomatosa* and the genetic composition of populations are important components to unravel for the conservation of the species (e.g. *Grevillea infecunda*, Kimpton, James and Drinnan 2002). Work is underway in these areas and will be reported elsewhere.

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